9/23/04

09/834,271

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     (FILE 'HOME' ENTERED AT 10:52:28 ON 23 SEP 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:53:12 ON 23 SEP 2004
L1        364623 S BACILLUS
L2           624 S "TATAAT" OR "TTGACA"
L3           114 S L1 AND L2
L4         50264 S "AMYQ" OR "ALPHA-AMYLASE"
L5            19 S L2 AND L4
L6            13 S L3 AND L4
L7             6 DUP REM L6 (7 DUPLICATES REMOVED)
L8           529 S "CRYIIIA"
L9           271 S "SP82"
L10          799 S L8 OR L9
L11           11 S L3 AND L10
L12            5 DUP REM L11 (6 DUPLICATES REMOVED)
                 E WIDNER W/AU
L13          110 S E3-E8
                 E SLOMA A/AU
L14          120 S E3
                 E THOMAS M D/AU
L15          393 S E3
L16          609 S L13 OR L14 OR L15
L17            5 S L3 AND L16
L18            4 DUP REM L17 (1 DUPLICATE REMOVED)

=>
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NEWS	10	AUG 27	BIOTECHABS/BIOTECHDS: Two new display fields added for legal status data from INPADOC
NEWS	11	SEP 01	INPADOC: New family current-awareness alert (SDI) available
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NEWS	14	SEP 14	STN Patent Forum to be held October 13, 2004, in Iselin, NJ

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=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci
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FILE 'LIFESCI' ENTERED AT 10:53:12 ON 23 SEP 2004
COPYRIGHT (C) 2004 Cambridge Scientific Abstracts (CSA)


=> s bacillus
L1      364623 BACILLUS

=> s "TATAAT" or "TTGACA"
L2         624 "TATAAT" OR "TTGACA"

=> s l1 and l2
L3         114 L1 AND L2

=> s "AMYQ" or "ALPHA-AMYLASE"
L4       50264 "AMYQ" OR "ALPHA-AMYLASE"

=> s l2 and l4
L5          19 L2 AND L4

=> s l3 and l4
L6          13 L3 AND L4

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7             6 DUP REM L6 (7 DUPLICATES REMOVED)

=> d 1-6 ibib ab

L7    ANSWER 1 OF 6  BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
      DUPLICATE 1
ACCESSION NUMBER: 2004-02033  BIOTECHDS
TITLE:            Generating an expression library of polynucleotides by
                  introducing the linear integration cassette into the host
                  cell and selecting or screening for host cells that produce
                  the polypeptides of interest;
                     protein library screening using homologous recombination
AUTHOR:           BJORNVAD M E; JORGENSEN P L; HANSEN P K
PATENT ASSIGNEE:  NOVOZYMES AS
```

PATENT INFO: WO 2003095658 20 Nov 2003
APPLICATION INFO: WO 2003-DK301 7 May 2003
PRIORITY INFO: DK 2002-682 7 May 2002; DK 2002-682 7 May 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
AB DERWENT ABSTRACT:
NOVELTY - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest.

DETAILED DESCRIPTION - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest. The cassette comprises: (1) a polynucleotide encoding one or more polypeptides of interest; (2) a 5' flanking polynucleotide segment upstream of the polynucleotide of (1) and comprising a first homologous region located in the 3' end of the segment; and (3) a 3' flanking polynucleotide segment downstream of the polynucleotide of (1) and comprising a second homologous region located in the 5' end of the segment. The first and second homologous regions are at least 500, 1000 or 1500 bp, each of which has a sequence identity of at least 80, 85, 90 or 95-100% with a region of the host cell genom. INDEPENDENT CLAIMS are also included for the following: (1) a non-replicating linear Gram-positive host cell integration cassette; and (2) a method of producing a polypeptide of interest.

BIOTECHNOLOGY - Preferred Method: Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises the additional step carried out between steps (1) and (2) that comprises introducing the plasmid into an intermediate Escherichia coli host cell and propagating it by replication. The integration cassette comprises: (1) an mRNA processing/stabilizing sequence derived from cryIIIa-gene; (2) a terminator downstream of the polynucleotide encoding the polypeptides of interest; and (3) a multiple cloning site with at least one recognition site for a restriction nuclease. It further comprises a marker gene located between the 5' and 3' flanking segments and at least one promoter that is a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region, and/or which is derived from amyL, **amyQ**, amyM, cryIIIA, dagA, aprH, penP, sacB, spoI, tac, xylA or xylB. The promoter is located between the flanking segments and is operably linked to the polynucleotide encoding one or more polypeptides of interest. Each of the 5' and 3' flanking polynucleotide segments comprises at least 500, 1000, 1500 or 2000 bp of non-homologous polynucleotides located in the 5' and 3' end of the 5' and 3' flanking segments, respectively. The promoter is one that results in that the host cells produce the polypeptides of interest in a yield of at least 10 mg/L. The polynucleotide comprises natural, synthetic or a library of shuffled or recombined homologs or variants of a gene or operon, provided by DNA breeding or DNA shuffling. The polypeptides of interest comprise enzymes, proteins or antimicrobial peptides. The enzymes are involved in the biosynthesis of hyaluronic acid. The Gram-positive host cell is **Bacillus** subtilis. The homologous region of the 5' and/or the 3' flanking segment is comprised in the yfmD-yfmC-yfmB-yfmA-pelB-yflS-citS region of the **Bacillus** subtilis genome or in the cryIIIa promoter. The non-replicating linear integration cassette is comprised in a plasmid and introduced into the host cell. The plasmid is capable of replicating in an Escherichia coli host cell but not in a **Bacillus** host cell. Producing a polypeptide of interest comprises culturing Gram-positive host cells

comprising the integration cassette integrated into its genome, under conditions promoting expression of the polypeptide of interest. The method further comprises isolating and/or purifying the polypeptide of interest.

USE - The method is useful in generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell (claimed). (55 pages)

L7 ANSWER 2 OF 6 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: THOMAS M D; SLOMA A
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]
AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), **Bacillus** clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis **alpha-amylase** gene (amyE), B. licheniformis **alpha-amylase** gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis CryIIIA gene (cryIIIA) or its portions, or preferably B. amyloliquefaciens **alpha-amylase** gene (**amyQ**). The mRNA processing/stabilizing sequence is the cryIIIA mRNA processing/stabilizing sequence. The **bacillus** cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence **TTGACA** for the 35 region, and **TATAAT** for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is

obtained from any bacterial or a **bacillus** promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L7 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a **Bacillus** cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a **Bacillus** host cell by introducing into a **Bacillus** cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a **Bacillus** cell by deleting a selectable marker gene of the **Bacillus** cell; and (4) a selectable marker-free mutant of a **Bacillus** cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained

from one or more **Bacillus** genes. The tandem promoter comprises the **amyQ** promoter, a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the **amyQ**, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the **Bacillus** cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The **Bacillus** cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the **Bacillus** cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the **Bacillus** cell. The **Bacillus** host cell is **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** brevis, **Bacillus** brevis, **Bacillus** circulans, **Bacillus** clausii, **Bacillus** coagulans, **Bacillus** firmus, **Bacillus** lautus, **Bacillus** lentus, **Bacillus** licheniformis, **Bacillus** megaterium, **Bacillus** pumilus, **Bacillus** sterothermophilus, **Bacillus** subtilis, or **Bacillus** thuringiensis. This method alternatively comprises cultivating a **Bacillus** cell in a medium conducive for the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a **Bacillus** promoter. Preferred Cell: The **Bacillus** cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a **Bacillus** cell, and for producing a selectable marker-free mutant of a **Bacillus** cell.

EXAMPLE - No relevant example given.(57 pages)

L7 ANSWER 4 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER:	1999:566201 HCAPLUS
DOCUMENT NUMBER:	131:180803
TITLE:	Nucleic acid vectors for recombinant production of heterologous proteins in a **Bacillus** cell
INVENTOR(S):	Widner, William; Sloma, Alan; Thomas, Michael D.
PATENT ASSIGNEE(S):	Novo Nordisk Biotech, Inc., USA
SOURCE:	PCT Int. Appl., 90 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English

FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412
PRIORITY APPLN. INFO.:			US 1998-31442 A	19980226
			US 1999-256377 B3	19990224
			WO 1999-US4360 W	19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as **amyQ** and amyL upstream of the cryIIIA promoter and its mRNA stabilizing sequence. Alternatively, "consensus" **amyQ** promoters are created with the cryIIIA mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus **amyQ** dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in **Bacillus** cells when compared to the levels obtained using single promoters such as **amyQ** and amyL.

L7 ANSWER 5 OF 6 MEDLINE on STN DUPLICATE 2

ACCESSION NUMBER:	92105008 MEDLINE
DOCUMENT NUMBER:	PubMed ID: 1370288
TITLE:	mRNA analysis of the adc gene region of Clostridium acetobutylicum during the shift to solventogenesis.
AUTHOR:	Gerischer U; Durre P
CORPORATE SOURCE:	Institut fur Mikrobiologie, Universitat Gottingen, Germany.
SOURCE:	Journal of bacteriology, (1992 Jan) 174 (2) 426-33. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY:	United States
DOCUMENT TYPE:	Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:	English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920302
Last Updated on STN: 19960129
Entered Medline: 19920212

AB By using primer extension analysis, we located the transcription start point of the acetoacetate decarboxylase (adc) gene of Clostridium acetobutylicum 90 nucleotides upstream from the initiation codon with A as the first transcribed nucleotide. From this site the promoter structure TTTACT(18 bp)**TATAAT** was identified; it shows high homology to the consensus sequences of gram-positive bacteria and Escherichia coli. Northern blot experiments revealed a length of 850 bases for the transcript of the adc gene. It thus represents a monocistronic operon. Transcription of adc was induced by conditions necessary for the onset of solvent formation. Induction occurred long before the respective fermentation product (acetone) could be detected in the medium. Transcription of the operon containing the genes for acetoacetyl coenzyme A:acetate/butyrate:coenzyme A transferase (designated ctf) downstream of the adc gene but divergently transcribed is also induced by conditions necessary for the onset of solvent formation. The length of the respective RNA transcript, 4.1 kb, indicates additional coding capacity, since the genes for the two subunits of the coenzyme A transferase cover only approximately 1.5 kb. No distinct transcripts for the other open reading frames of the adc gene region, ORF1 and ORF2, could be detected. Computer analysis indicated that ORF1, which showed significant similarity to the **alpha-amylase** gene of **Bacillus** subtilis (U. Gerischer and P. Durre, J. Bacteriol. 172:6907-6918, 1990), probably is indeed a coding region. ORF2, however, does not seem to have a coding function.

L7 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 90362027 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2391488
TITLE: Nucleotide sequence of the **alpha-amylase** -pullulanase gene from Clostridium thermohydrosulfuricum.
AUTHOR: Melasniemi H; Paloheimo M; Hemio L
CORPORATE SOURCE: Research Laboratories, Alko Ltd., Helsinki, Finland.
SOURCE: Journal of general microbiology, (1990 Mar) 136 (Pt 3) 447-54.
Journal code: 0375371. ISSN: 0022-1287.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M28471
ENTRY MONTH: 199010
ENTRY DATE: Entered STN: 19901109
Last Updated on STN: 19901109
Entered Medline: 19901001

AB The nucleotide sequence of the gene (apu) encoding the thermostable **alpha-amylase**-pullulanase of Clostridium thermohydrosulfuricum was determined. An open reading frame of 4425 bp was present. The deduced polypeptide (Mr 165,600), including a 31 amino acid putative signal sequence, comprised 1475 amino acids, with no cysteine residues. The structural gene was preceded by the consensus promoter sequence **TTGACA TATAAT**, a putative regulatory sequence and a putative ribosome-binding sequence AAAGGGGG. The codon usage resembled that of **Bacillus** genes. The deduced sequence of the mature apu product showed similarities to various amylolytic enzymes, especially the neopullulanase of **Bacillus** stearothermophilus, whereas the signal sequence showed similarity to those of the **alpha-amylases** of B. stearothermophilus and B. subtilis. Three regions thought to be highly conserved in the primary structure of **alpha-amylases** could also be distinguished in the apu

product, two being partly 'duplicated' in this alpha-1,4/alpha-1,6-active enzyme.

```
=> s "CRYIIIA"
L8          529 "CRYIIIA"

=> s "SP82"
L9          271 "SP82"

=> s l8 or l9
L10         799 L8 OR L9

=> s l3 and l10
L11          11 L3 AND L10

=> dup rem l11
PROCESSING COMPLETED FOR L11
L12            5 DUP REM L11 (6 DUPLICATES REMOVED)

=> d 1-5 ibib ab

L12   ANSWER 1 OF 5  BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
      DUPLICATE 1
ACCESSION NUMBER: 2004-02033  BIOTECHDS
TITLE:            Generating an expression library of polynucleotides by
                  introducing the linear integration cassette into the host
                  cell and selecting or screening for host cells that produce
                  the polypeptides of interest;
                     protein library screening using homologous recombination
AUTHOR:           BJORNVAD M E; JORGENSEN P L; HANSEN P K
PATENT ASSIGNEE:  NOVOZYMES AS
PATENT INFO:      WO 2003095658 20 Nov 2003
APPLICATION INFO: WO 2003-DK301 7 May 2003
PRIORITY INFO:    DK 2002-682 7 May 2002; DK 2002-682 7 May 2002
DOCUMENT TYPE:    Patent
LANGUAGE:         English
AB    DERWENT ABSTRACT:
      NOVELTY - Generating an expression library of polynucleotides integrated
      by homologous recombination into the genome of a competent Gram-positive
      bacterium host cell comprises: (a) providing a non-replicating linear
      integration cassette; and (b) introducing the linear integration cassette
      into the host cell and selecting or screening for host cells that produce
      the polypeptides of interest.
           DETAILED DESCRIPTION - Generating an expression library of
      polynucleotides integrated by homologous recombination into the genome of
      a competent Gram-positive bacterium host cell comprises: (a) providing a
      non-replicating linear integration cassette; and (b) introducing the
      linear integration cassette into the host cell and selecting or screening
      for host cells that produce the polypeptides of interest. The cassette
      comprises: (1) a polynucleotide encoding one or more polypeptides of
      interest; (2) a 5' flanking polynucleotide segment upstream of the
      polynucleotide of (1) and comprising a first homologous region located in
      the 3' end of the segment; and (3) a 3' flanking polynucleotide segment
      downstream of the polynucleotide of (1) and comprising a second
      homologous region located in the 5' end of the segment. The first and
      second homologous regions are at least 500, 1000 or 1500 bp, each of
      which has a sequence identity of at least 80, 85, 90 or 95-100% with a
      region of the host cell genom. INDEPENDENT CLAIMS are also included for
      the following: (1) a non-replicating linear Gram-positive host cell
      integration cassette; and (2) a method of producing a polypeptide of
      interest.
           BIOTECHNOLOGY - Preferred Method: Generating an expression library
      of polynucleotides integrated by homologous recombination into the genome
```

of a competent Gram-positive bacterium host cell comprises the additional step carried out between steps (1) and (2) that comprises introducing the plasmid into an intermediate Escherichia coli host cell and propagating it by replication. The integration cassette comprises: (1) an mRNA processing/stabilizing sequence derived from **cryIIIa**-gene; (2) a terminator downstream of the polynucleotide encoding the polypeptides of interest; and (3) a multiple cloning site with at least one recognition site for a restriction nuclease. It further comprises a marker gene located between the 5' and 3' flanking segments and at least one promoter that is a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region, and/or which is derived from amyL, amyQ, amyM, **cryIIIA**, dagA, aprH, penP, sacB, spol, tac, xylA or xylB. The promoter is located between the flanking segments and is operably linked to the polynucleotide encoding one or more polypeptides of interest. Each of the 5' and 3' flanking polynucleotide segments comprises at least 500, 1000, 1500 or 2000 bp of non-homologous polynucleotides located in the 5' and 3' end of the 5' and 3' flanking segments, respectively. The promoter is one that results in that the host cells produce the polypeptides of interest in a yield of at least 10 mg/L. The polynucleotide comprises natural, synthetic or a library of shuffled or recombined homologs or variants of a gene or operon, provided by DNA breeding or DNA shuffling. The polypeptides of interest comprise enzymes, proteins or antimicrobial peptides. The enzymes are involved in the biosynthesis of hyaluronic acid. The Gram-positive host cell is **Bacillus** subtilis. The homologous region of the 5' and/or the 3' flanking segment is comprised in the yfmD-yfmC-yfmB-yfmA-pelB-yflS-citS region of the **Bacillus** subtilis genome or in the **cryIIIa** promoter. The non-replicating linear integration cassette is comprised in a plasmid and introduced into the host cell. The plasmid is capable of replicating in an Escherichia coli host cell but not in a **Bacillus** host cell. Producing a polypeptide of interest comprises culturing Gram-positive host cells comprising the integration cassette integrated into its genome, under conditions promoting expression of the polypeptide of interest. The method further comprises isolating and/or purifying the polypeptide of interest.

USE - The method is useful in generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell (claimed). (55 pages)

L12 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: THOMAS M D; SLOMA A
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]
AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), **Bacillus** clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis **CryIIIA** gene (**cryIIIA**) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (amyQ). The mRNA processing/stabilizing sequence is the **cryIIIA** mRNA processing/stabilizing sequence. The **bacillus** cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence **TTGACA** for the 35 region, and **TATAAT** for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a **bacillus** promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L12 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003

APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]

AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a **Bacillus** cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a **Bacillus** host cell by introducing into a **Bacillus** cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a **Bacillus** cell by deleting a selectable marker gene of the **Bacillus** cell; and (4) a selectable marker-free mutant of a **Bacillus** cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more **Bacillus** genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** from the -10 region, the amyL promoter, and/or the **cryIIIA** promoter. The tandem promoter comprises two copies of the amyQ, amyl or **cryIIIA** promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the **Bacillus** cell. The mRNA processing/stabilizing sequence is the **cryIIIA** or **SP82** mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The **Bacillus** cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the **Bacillus** cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the **Bacillus** cell. The **Bacillus** host cell is **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** brevis, **Bacillus** brevis, **Bacillus** circulans, **Bacillus** clausii, **Bacillus** coagulans, **Bacillus** firmus, **Bacillus** lautus, **Bacillus** lentus, **Bacillus** licheniformis, **Bacillus** megaterium, **Bacillus** pumilus,

Bacillus sterothermophilus, **Bacillus** subtilis, or **Bacillus** thuringiensis. This method alternatively comprises cultivating a **Bacillus** cell in a medium conducive for the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a **Bacillus** promoter. Preferred Cell: The **Bacillus** cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a **Bacillus** cell, and for producing a selectable marker-free mutant of a **Bacillus** cell.

EXAMPLE - No relevant example given.(57 pages)

L12 ANSWER 4 OF 5 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2001393416 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11234961
TITLE: Construction of protein overproducer strains in **Bacillus** subtilis by an integrative approach.
AUTHOR: Jan J; Valle F; Bolivar F; Merino E
CORPORATE SOURCE: Departamento de Microbiologia Molecular, Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Morelos.
SOURCE: Applied microbiology and biotechnology, (2001 Jan) 55 (1) 69-75.
Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010716
Last Updated on STN: 20010716
Entered Medline: 20010712

AB We evaluated the effect of several genetic factors reported as having a role in the induction of the expression of significant levels of recombinant protein in **Bacillus** subtilis. We utilized the beta-galactosidase reporter protein from Escherichia coli as our model for measuring the overproduction of heterologous proteins in B. subtilis. The lacZ gene was expressed in B. subtilis using the regulatory region of the subtilisin gene aprE. In this study, we considered factors known to modulate the transcription and translation initiation rates and genetic and mRNA stability. We also consider the effects of different genetic backgrounds, such as degU32 and hpr2, that until now have been studied independently. By changing the native -35 promoter box to the consensus **TTGACA** sequence of the aprE promoter, a significant 100-fold increase in the beta-galactosidase activity was obtained. On the other hand, changes such as the GTG to ATG start codon, the construction of a consensus AAGGAGG ribosome binding site, and the addition of the **cryIIIA** transcription terminator at the 3' end of the lacZ gene, produced only marginal effects on the final beta-galactosidase activity.

L12 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a **Bacillus** cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.

PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412

PRIORITY APPLN. INFO.:	US 1998-31442	A	19980226
	US 1999-256377	B3	19990224
	WO 1999-US4360	W	19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the **cryIIIA** promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the **cryIIIA** mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in **Bacillus** cells when compared to the levels obtained using single promoters such as amyQ and amyL.

=> d his

(FILE 'HOME' ENTERED AT 10:52:28 ON 23 SEP 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:53:12 ON 23 SEP 2004

```
L1        364623 S BACILLUS
L2           624 S "TATAAT" OR "TTGACA"
L3           114 S L1 AND L2
L4         50264 S "AMYQ" OR "ALPHA-AMYLASE"
L5            19 S L2 AND L4
L6            13 S L3 AND L4
L7             6 DUP REM L6 (7 DUPLICATES REMOVED)
L8           529 S "CRYIIIA"
L9           271 S "SP82"
L10          799 S L8 OR L9
L11           11 S L3 AND L10
L12            5 DUP REM L11 (6 DUPLICATES REMOVED)

=> e widner w/au
E1            32     WIDNER T E/AU
E2             2     WIDNER THOMAS E/AU
E3            30 --> WIDNER W/AU
E4             7     WIDNER W E/AU
E5            41     WIDNER W R/AU
E6            13     WIDNER WILLIAM/AU
E7            18     WIDNER WILLIAM R/AU
E8             1     WIDNER WILLIAM ROY/AU
E9             1     WIDNER WM R/AU
E10            2     WIDNES J/AU
E11            2     WIDNES J A/AU
E12            2     WIDNES V/AU

=> s e3-e8
L13          110 ("WIDNER W"/AU OR "WIDNER W E"/AU OR "WIDNER W R"/AU OR "WIDNER
                 WILLIAM"/AU OR "WIDNER WILLIAM R"/AU OR "WIDNER WILLIAM ROY"/AU)


=> e sloma a/au
E1             3     SLOM T J/AU
E2             2     SLOM TREVOR J/AU
E3           120 --> SLOMA A/AU
E4            15     SLOMA A P/AU
E5            54     SLOMA ALAN/AU
E6            12     SLOMA ALAN P/AU
E7             1     SLOMA ALAN PAUL/AU
E8             1     SLOMA D/AU
E9             1     SLOMA D R/AU
E10            6     SLOMA E/AU
E11            2     SLOMA E J/AU
E12            2     SLOMA J/AU

=> s e3
L14          120 "SLOMA A"/AU

=> e thomas m d/au
E1             4     THOMAS M C C/AU
E2            12     THOMAS M CARMEN/AU
E3           393 --> THOMAS M D/AU
E4            95     THOMAS M D A/AU
E5             4     THOMAS M D H/AU
E6             3     THOMAS M D JR/AU
E7             2     THOMAS M D O/AU
E8            13     THOMAS M D R/AU
E9           331     THOMAS M E/AU
E10           33     THOMAS M E A/AU
E11            3     THOMAS M E C/AU
E12           32     THOMAS M E M/AU

=> s e3
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```
L15         393 "THOMAS M D"/AU

=> s l13 or l14 or l15
L16         609 L13 OR L14 OR L15

=> d his

     (FILE 'HOME' ENTERED AT 10:52:28 ON 23 SEP 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:53:12 ON 23 SEP 2004
L1       364623 S BACILLUS
L2          624 S "TATAAT" OR "TTGACA"
L3          114 S L1 AND L2
L4        50264 S "AMYQ" OR "ALPHA-AMYLASE"
L5           19 S L2 AND L4
L6           13 S L3 AND L4
L7            6 DUP REM L6 (7 DUPLICATES REMOVED)
L8          529 S "CRYIIIA"
L9          271 S "SP82"
L10         799 S L8 OR L9
L11          11 S L3 AND L10
L12           5 DUP REM L11 (6 DUPLICATES REMOVED)
                E WIDNER W/AU
L13         110 S E3-E8
                E SLOMA A/AU
L14         120 S E3
                E THOMAS M D/AU
L15         393 S E3
L16         609 S L13 OR L14 OR L15

=> s l3 and l16
L17           5 L3 AND L16

=> dup rem l17
PROCESSING COMPLETED FOR L17
L18           4 DUP REM L17 (1 DUPLICATE REMOVED)

=> d 1-4 ibib ab

L18   ANSWER 1 OF 4  BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08483  BIOTECHDS
TITLE:            Production of a secreted polypeptide having L-asparaginase
                  activity for treating leukemia, comprises cultivating a host
                  cell comprising a nucleic acid having a sequence encoding a
                  secretory signal peptide linked to a second sequence;
                     vector-mediated enzyme gene transfer and expression in
                     host cell for recombinant protein production, amino acid
                     preparation and disease therapy
AUTHOR:           THOMAS M D; SLOMA A
PATENT ASSIGNEE:  NOVOZYMES BIOTECH INC
PATENT INFO:      US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO:    US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2004-088916 [09]
AB    DERWENT ABSTRACT:
      NOVELTY - Producing a secreted polypeptide having L-asparaginase
      activity, comprises cultivating a host cell containing a nucleic acid
      construct having a sequence encoding a secretory signal peptide linked to
      a second sequence encoding the polypeptide having L-asparaginase
      activity, where the signal peptide directs the polypeptide into the
      cell's secretory pathway, and recovering the secreted polypeptide.
```

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), **Bacillus** clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis CryIIIA gene (cryIIIA) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (amyQ). The mRNA processing/stabilizing sequence is the cryIIIA mRNA processing/stabilizing sequence. The **bacillus** cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence **TTGACA** for the 35 region, and **TATAAT** for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a **bacillus** promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L18 ANSWER 2 OF 4 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: **WIDNER W; SLOMA A; THOMAS M D**
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003

APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]

AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a **Bacillus** cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a **Bacillus** host cell by introducing into a **Bacillus** cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a **Bacillus** cell by deleting a selectable marker gene of the **Bacillus** cell; and (4) a selectable marker-free mutant of a **Bacillus** cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more **Bacillus** genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the **Bacillus** cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The **Bacillus** cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the **Bacillus** cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the **Bacillus** cell. The **Bacillus** host cell is **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** brevis, **Bacillus** brevis, **Bacillus** circulans, **Bacillus** clausii, **Bacillus** coagulans, **Bacillus** firmus, **Bacillus** lautus, **Bacillus** lentus, **Bacillus** licheniformis, **Bacillus** megaterium, **Bacillus** pumilus, **Bacillus** sterothermophilus,

Bacillus subtilis, or **Bacillus** thuringiensis. This method alternatively comprises cultivating a **Bacillus** cell in a medium conducive for the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a **Bacillus** promoter. Preferred Cell: The **Bacillus** cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a **Bacillus** cell, and for producing a selectable marker-free mutant of a **Bacillus** cell.

EXAMPLE - No relevant example given.(57 pages)

L18 ANSWER 3 OF 4 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
DUPLICATE 1

ACCESSION NUMBER: 2001:378829 BIOSIS
DOCUMENT NUMBER: PREV200100378829
TITLE: Methods for producing a polypeptide in a **Bacillus** cell.
AUTHOR(S): **Widner, William** [Inventor, Reprint author]; Sloma, Alan [Inventor]; Thomas, Michael D. [Inventor]
CORPORATE SOURCE: Davis, CA, USA
ASSIGNEE: Novozymes Biotech, Inc., Davis, CA, USA
PATENT INFORMATION: US 6255076 July 03, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 3, 2001) Vol. 1248, No. 1. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Aug 2001
Last Updated on STN: 19 Feb 2002

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium.

L18 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of

heterologous proteins in a **Bacillus** cell

INVENTOR(S): **Widner, William**; Sloma, Alan; Thomas, Michael D.

PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA

SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412

PRIORITY APPLN. INFO.:

US 1998-31442	A	19980226
US 1999-256377	B3	19990224
WO 1999-US4360	W	19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the cryIIIA promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the cryIIIA mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in **Bacillus** cells when compared to the levels obtained using single promoters such as amyQ and amyL.

=> d his

(FILE 'HOME' ENTERED AT 10:52:28 ON 23 SEP 2004)

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:53:12 ON 23 SEP 2004
L1        364623 S BACILLUS
L2           624 S "TATAAT" OR "TTGACA"
L3           114 S L1 AND L2
L4         50264 S "AMYQ" OR "ALPHA-AMYLASE"
L5            19 S L2 AND L4
L6            13 S L3 AND L4
L7             6 DUP REM L6 (7 DUPLICATES REMOVED)
L8           529 S "CRYIIIA"
L9           271 S "SP82"
L10          799 S L8 OR L9
L11           11 S L3 AND L10
L12            5 DUP REM L11 (6 DUPLICATES REMOVED)
                 E WIDNER W/AU
L13          110 S E3-E8
                 E SLOMA A/AU
L14          120 S E3
                 E THOMAS M D/AU
L15          393 S E3
L16          609 S L13 OR L14 OR L15
L17            5 S L3 AND L16
L18            4 DUP REM L17 (1 DUPLICATE REMOVED)
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